Dear Editor,

Thank you very much for decision letter along with the reviewer’s comments for our manuscript No.: ijms-414213

The reviewers requested modification, and more clarification. They also gave us several excellent suggestions which would strengthen our manuscript. We thank for reviewer’s constructive criticisms.

Please find some of our changes in revised MS. as highlighted in red in marked version.

Our point by point response to the comments of the reviewer is as follows:

Reviewer 1:

1. In general, the mechanistic study of this MS is not deep despite many data. There are lacking evidences for title. How about effect of mTOR/p70S6K/4E-BP1 and Raf/MEK/ERK inhibitor or siRNA transfection on proliferation and HIF1 alpha in HCT116 cells under hypoxia?

Response 1: Thanks to the reviewer’s suggestions. Previously, there are many papers that have proved that mTOR/p70S6K/4E-BP1 and Raf/MEK/ERK inhibitor or siRNA transfection can reduced the proliferation and HIF-1α expression [1-3]. The aim of our article is to study nature products which have potential anti-cancer effects and we have proved that vanillic acid could be a profound lead compound for further study. In addition, we have referenced articles that study the effect of drugs on proliferation and HIF-1α signaling pathway, while these articles have not study the effect of mTOR/p70S6K/4E-BP1 and Raf/MEK/ERK inhibitor or siRNA on proliferation and HIF-1α expression in cancer cells [4-7]. We will exam the mTOR/p70S6K/4E-BP1
and Raf/MEK/ERK inhibitor or siRNA for in-depth mechanism study in the next paper when we contribute to your journal.

The references are as below.


2. Show antiproliferative effect of vanillic acid in other colon cancer lines under hypoxia. You just showed its effect only on HCT116 cells?

Response 2: We thank the reviewer’s suggestions. Refer to the following articles, using singly HCT116 cell line might adequately prove the anti-proliferative effect of active compound [1-3], and HCT116 cells is a representative and common used cell line for the research of colon cancer.

In addition, referred to the title of the following articles, we have revised the title “vanillic acid suppresses HIF-1α expression via inhibition of mTOR/p70S6K/4E-BP1 and Raf/MEK/ERK pathways in human colon cancer cells” to “Vanillic acid suppresses HIF-1α expression via inhibition of mTOR/p70S6K/4E-BP1 and Raf/MEK/ERK pathways in human colon cancer HCT116 cells” [4-6]. For above reasons, we chose HCT116 cells for subsequent assays in our study.

The references are as below.
4. Wang, H. G.; Cao, B.; Zhang, L. X.; Song, N.; Li, H.; Zhao, W. Z.; Li, Y. S.; Ma, S.


3. Add significance between lane 4 and lane 4 in Fig3 F.

Response 3: We thank the reviewer’s suggestions. The intensity of the protein bands from the western blot experiments were quantified again. As shown in Fig3 F, lane 4 have significant differences compared with lane 2. We have revised the figures as below:

4. Method of tube formation assay is not clear. Did you add media supernatant on HUVEcs?
Response 4: We thank the reviewer’s suggestions. In the tube formation assay, we added DMEM medium on HUVEC cells. In other words, HUVEC cells suspended in 300 μl of fresh DMEM medium were added to each well coated with matrigel and treated with or without vanillic acid for 12 h. We have added the method of tube formation assay in the manuscript. We have revised the method as below:

We have revised “Chilled liquid matrigel was dispensed onto 24-well plates and allowed to solidify. Then HUVEC cells were seeded onto the gel and cultured in the medium containing vanillic acid at 37°C for 12 h. Matrigel was fixed, and examined under inverted microscope (Olympus, Tokyo, Japan)” to “Chilled liquid Matrigel was dispensed onto 24-well plates (300 μl per well), and allowed to solidify for 1 h at 37 °C according to the manufacturer’s instructions. HUVEC cells (2.5 × 10⁴ cells/well) suspended in 300 μl of fresh DMEM medium were added to each well coated with matrigel and treated with or without vanillic acid for 12 h. Tube formation and capillary tube lengths were observed under a microscope and photographed (Olympus, Tokyo, Japan)” (Page 4, Line 154 to 158).

5. Rewrite Abstract including G1 arrest and VEGF data.

Response 5: We thank the reviewer’s suggestions. We have rewrited abstract including G1 arrest and VEGF data in the manuscript. We have revised the contents as below:

We have revised “Furthermore, we found that vanillic acid inhibited angiogenesis by down regulating vascular endothelial growth factor and erythropoietin expression. Moreover, vanillic acid inhibited cell proliferation via blocking cell cycle progression” to “We found that vanillic acid dose-dependently inhibited VEGF and EPO protein expressions and disrupt tube formation. The results suggest that vanillic acid effectively inhibits angiogenesis. Flow cytometry analysis demonstrated that vanillic acid significantly induced G1 phase arrest and inhibited the proliferation of HCT116 cells” (Page 1, Line 23 to 27).